PE Examination of Evidence with a Compound Microscope

A. SCOPE

A.1 Optical microscopy employing the compound microscope is a broad topic in which a diverse array of analytical approaches can be potentially performed. Compound microscopes typically have multiple objective lenses which can be rotated into the field of view providing magnification levels ranging between 40 and 1000x; they are powerful enough to view hairs, fibers, and cells. This document will address only those subjects which are directly pertinent to the examination of evidence typically encountered in the Primary Examination Section. Here, the compound microscope can be used for the identification of spermatozoa and for the determination of the possible suitability of a hair root for DNA analysis. The compound microscopes used for these examinations in the Primary Examination Section are Leica DMLB and Leica DM2500 models. Each is able to allow for specimen viewing by bright field microscopy or phase contrast microscopy. Bright field microscopy is the simplest form of imaging a specimen; this technique takes the specimen which is dark and contrasts it by the surrounding bright viewing field. Phase contrast microscopy is most useful in viewing "phase objects" which are transparent and colorless; this technique shows differences in refractive index as differences in contrast.

B. QUALITY CONTROL

B.1 Not applicable

C. SAFETY

C.1 Treat all biological samples as potentially infectious; employ appropriate safety protocols.

C.2 Ensure the illumination intensity setting is at a low level before initially looking through the microscope then adjust the intensity for comfortable viewing.

C.3 Do not open a lamp housing until the lamp has had adequate time to completely cool.

C.4 Do not cover a hot lamp housing. Allow the lamp to cool before covering with the instrument's dust cover.

D. REAGENTS, STANDARDS AND CONTROLS

D.1 70% ethanol solution (decontamination)

E. EQUIPMENT

E.1 Leica DMLB microscope

E.2 Leica DM2500 microscope

F. PROCEDURES

F.1 Rotate the desired objective (4x, 10x, or 40x) into the light path.

F.2 For bright field viewing, rotate the turret wheel in the condenser to bring an empty accessory space into place (marked "BF" for bright field). For phase contrast viewing adjust the turret wheel to bring into place the appropriate phase annular ring for the objective being used, i.e. "Ph2" for "Ph2" etc.

F.3 Adjust the brightness of the light source by using the light intensity control on the unit.

F.4 Place the slide on the mechanical stage.

F.5 Use the slide holder to gently secure the slide.

F.6 Turn the X and Y stage knobs to position the specimen in the center of the viewing field.

F.7 Adjust the interpupillary distance for the eyepieces by pushing the eyepieces together or apart. Interpupillary distance varies from person to person; therefore, each person should make this adjustment before using the microscope for the best quality image. The interpupillary distance is correct when the left and right fields of view converge completely into one image.

F.8 Dioptric correction is used to compensate for the difference between a person's eyes. Each user can adjust the diopter settings prior to using the compound microscope to reduce eye strain. To adjust the diopter settings, first rotate the diopter rings on the eyepiece tubes until their numerical value is the same as your interpupillary distance. Close your left eye and bring the specimen into focus using the focusing knobs. Then close your right eye and bring the specimen into focus by adjusting the diopter ring on the left eyepiece tube only; do not use the focus knobs at this step.

F.9 With the desired objective in position, raise the mechanical stage using the coarse focus knob until the specimen is close to the objective.

F.10 Turn the coarse focus knob until the specimen is in focus.

F.11 Use the fine focus knob to obtain a sharp image.

F.12 Adjust the magnification to the desired level by rotating the objectives.

F.13 Upon completion of your examination, turn down the light intensity and then shut off the power.

F.14 The body of the compound microscope can be cleaned using either a 70% ethanol solution or warm soapy water, followed by a wipe down with distilled water. The eyepiece and objective lenses can be cleaned with compressed air followed by lens paper moistened with an optic cleaning solution, if necessary.

F.15 A dust cover should be placed over the compound microscope when it is not in use.

F.16 Should the lamp on a compound microscope, need to be replaced, the service provider may be contacted.

F.17 If an analyst determines that the compound microscope is out of alignment and/or not at optimal viewing conditions, they may choose to re-setup the microscope for Köhler illumination and if necessary, phase contrast viewing. Methods for proper critical

illumination and phase contrast viewing can be found in the References section of this document.

G. INTERPRETATION GUIDELINES

G.1 Not applicable

H. REFERENCES

H.1 Leica DML Instructions, Leica Microsystems, Wetzlar, Germany, 1998.

H.2 Leica DM2000, Leica DM2500, Leica DM3000 Operating Manual, Leica Microsystems, Wetzlar, Germany, 2006.

H.3 Microscope- Basics and Beyond, URL micro.magnet.fsu.edu/primer/pdfs/basicsandbeyond.pdf

H.4 Köhler Microscope Illumination, URL micro.magnet.fsu.edu/primer/anatomy/kohler.html

H.5 Introduction to Phase Contrast, URL micro.magnet.fsu.edu/primer/techniques/phasecontrast/phase.html

H.6 Phase Contrast Microscope Configuration, URL microscopyu.com/articles/phasecontrast/phaseconfiguration.html

H.7 Procedure for Critical Viewing (Köhler Illumination), Rich Berger, February 2013.

H.8 Procedure for Phase Contrast Viewing, Rich Berger, February 2013.